TISSUE MODIFICATIONS IN MONKEYS AS RELATED TO ABSORPTION, DISTRIBUTION, AND EXCRETION OF POLYCHLORINATED BIPHENYLS

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Adult rhesus monkeys were given a single dose of 1.5 or 3.0 g of a polychlorinated biphenyl (PCB) (Arochlor 1248) per kg body weight and sacrificed four days later. In addition, one monkey from each group was placed in a metabolism cage and the excreta collected during a two-week period and analyzed for PCB content. Over 90 percent of the single PCB dosage was absorbed from the gastrointestinal tract and deposited in various tissues of the body. Elimination of the PCBs, primarily through the biliary system, occurred at a slow rate. The monkeys did not become obviously ill following ingestion of the PCBs, but developed moderate hepatic enlargement due primarily to an increase in lipid droplets and proliferation of the endoplasmic reticulum of the hepatic cells. Gastric hypertrophy and hyperplasia and focal ulceration of the stomach lining were prominent lesions in these animals.

The polychlorinated biphenyls (PCBs) have been used extensively for various industrial purposes during the past 40 years. Because of their chemical inertness these compounds are highly resistant to degradation in the environment. Through industrial accidents and improper disposal and usage, they have become global environmental contaminants. Ingestion of minute amounts of the PCBs have produced widespread deleterious effects on the wildlife population throughout the world (Holmes *et al.* 1967; Jensen *et al.* 1969; Koeman *et al.* 1967, 1969). Industrial accidents leading to ingestion by man have led to serious illness and long-lasting morbidity (Kuratsune *et al.* 1972). The presently reported study was conducted to determine acute toxic effects on nonhuman primates of PCBs (Aroclor 1248[1]) and to evaluate absorption, tissue levels, and routes of excretion of these compounds following exposure to a single dosage.

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[1]Monsanto Company, St. Louis, Missouri.

Materials and methods

Seventeen male rhesus monkeys, ranging in age from two to 2.5 years and weighing between three and 3.5 kg, were placed in three groups. The two experimental groups consisting of six animals per group were given 1.5 or 3.0 g of a PCB (Aroclor 1248) per kg body weight by gastric intubation. The third group of five animals served as controls. All of the experimental animals were deprived of food for one day prior to intubation. Following the administration of the PCBs the animals were allowed to eat *ad libitum* for three days, after which five animals in each experimental group received no food for one day and were subsequently sacrificed. Blood studies including evaluation of white blood cells (WBC), hemoglobin, hematocrit, differential white cell counts, serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) (Reitman and Frankel 1957), total protein (Werchselbaum 1946), serum protein electrophoresis (Beckman, 1965), and blood urea nitrogen (BUN) (Bohoun *et al.* 1968) were done both prior to intubation and before each animal was sacrificed. Prior to death the animals were anesthetized, the jugular vessels severed, and the animals allowed to exsanguinate. Tissues were obtained immediately for microscopic and biochemical studies. For histological evaluations, small sections of the tissues were placed in ten percent neutral buffered formalin for 24 hr. They were subsequently dehydrated, embedded in paraffin, sectioned at five microns, and stained with hematoxylin and eosin (Armed Forces Institute of Pathology 1960). The hepatic tissues employed in the electron microscopic studies were cut into small cubes and fixed in osmium tetroxide and buffered with veronal acetate (Caulfield 1957) for 1.5 hr. They were subsequently dehydrated through a graded series of ethanol and embedded in an epoxy resin mixture (Mollenhauer 1964). Sections of the tissues were cut on an ultramicrotome, placed on uncoated copper grids, stained with uranyl acetate, and examined with an electron microscope.

Additional portions of the liver were homogenized at 0°C with two volumes of 0.25 M sucrose with 0.010 M $MgCl_2$ and 0.015 M KCl (SKM). Levels of protein (Lowry *et al.* 1951) and DNA and RNA (Munro and Fleck 1966) were determined on portions of the homogenate. Microsomes were isolated by centrifugation at 100,000 G for 90 min of the postmitochondrial supernatant (which was obtained by centrifugation at 9,000 G for 20 min), washed by homogenization with sucrose and recentrifuged, then resuspended in SKM equal to three volumes of liver and stored at −70°C. The activities of aromatic hydroxylase, nitroreductase, *N*-demethylase, esterase, and glucose-6-phosphatase and concentrations of protein were determined by methods previously reported (Norback and Allen 1972).

At the beginning of the experiment one animal from each of the two treatment levels was placed in a metabolism cage and the urine and feces collected prior to intubation and over a two-week period thereafter. In addition to the urine and feces from these two animals, the brain, liver, and kidneys were also obtained from all animals and analyzed for content of PCBs. Liver and kidney samples were homogenized in hexane (500 mg: 20 ml); urine was extracted in hexane (10 ml: 15 ml); and feces were desiccated over $CaCl_2$ for one week, pulverized in a mortar and pestle, and extracted in acetone (1 gm:25 ml). Brain tissue was extracted in acetone (500 mg:20 ml) (Jennings 1968) and

all tissue extracts were subsequently evaporated under dry nitrogen with anhydrous sodium sulfate in a 40°C water bath. Clean-up of the extracted material prior to analysis was carried out in a disposable pipet microcolumn (Curley *et al.* 1971) containing silica gel 60 (0.05-0.2 mm) by elution with a 1:1 benzene-hexane mixture. Levels of PCBs were quantitated in a Hewlett-Packard Research (7000) Chromatograph employing a ^{63}Ni electron capture detector. The Pyrex glass column packed with Gas Chrom Q (80-100 mesh) coated with two percent SE-30 was operated at 170°C with argon-methane (95%-5%) as carrier gas at a flow rate of 40 cc/min.

PCB recovery studies were conducted on control samples of tissue and excreta. The PCBs were added directly to the urine, dried feces, and tissue homogenates and subsequently handled in a manner similar to that previously described.

Results

Following the administration of 1.5 or 3.0 g per kg of body weight of the PCB mixture, Aroclor 1248, to the experimental monkeys, there were no gross manifestations of illness during the subsequent four days and the general appearance of the animals remained unchanged. Body weight of the animals immediately before receiving the PCBs (3.14 kg ± 0.40 for the higher dosage group; 3.03 kg ± 0.33 for the lower dosage group) did not differ significantly from the body weight four days after PCB administration (3.02 kg ± 0.46 for the higher dosage group; 2.97 ± 0.28 for the lower dosage group). The animals that were allowed to survive for 14 days became increasingly anorectic and lethargic and at 14 days weighed 2.7 kg (after receiving 1.5 g PCBs/kg) and 1.8 kg (after receiving 3.0 g PCBs/kg). As shown in Table I the experimental animals had slight but significant decreases in hemoglobin and hematocrit levels. The moderate leukocytosis was attributed primarily to an increase in neutrophils. There was also a slight rise in the level of SGOT, while the levels of SGPT were moderately reduced. The total serum protein level was slightly decreased. Levels of BUN were not modified appreciably.

Gross lesions of the external surface of the body or of the internal organs were absent at sacrifice on the fourth day following administration of the PCBs. Microscopic examination of the tissues revealed a moderate hypertrophy and hyperplasia of the gastric mucosa and the presence of isolated mucinous cysts within the epithelium of the stomach. In addition, there was an occasional penetration of the muscularis mucosae and the invasion of the submucosa by isolated glandular elements of the mucosal epithelium. There was also moderate edema of the submucosa of the stomach. These gastric changes were markedly accentuated in the two animals that were housed in metabolism cages and allowed to survive for two weeks. In these animals, the major changes in the stomach were associated with hypertrophy and hyperplasia of the gastric mucosa. There were large collections of gastric mucosal epithelium that had penetrated the muscularis mucosae forming large cysts and sheets of glandular epithelium throughout the edematous submucosa. In addition, segments of the hyperplastic gastric mucosa were eroded thereby forming distinct ulcers.

Table I. *Hematological Changes in Monkeys Four Days Following PCB (Aroclor 1248[a]) Administration*

Assays conducted	Control[b]	PCB dosage[b] (3 g/kg)	PCB dosage[b] (1.5 g/kg)
Hg (gm%)	13.8 ± 0.9	12.2 ± 0.6[c]	11.9 ± 0.6[c]
Hct (%)	43.1 ± 3.2	39.0 ± 1.9[d]	37.4 ± 1.7[d]
WBC (10^3/cmm)	6.3 ± 1.8	9.9 ± 2.4	9.8 ± 3.0
Neutrophils (%)	44.5 ± 12.0	63.0 ± 16.0	63.0 ± 14.8[e]
Lymphocytes (%)	54.4 ± 12.0	37.0 ± 15.2	36.0 ± 14.6[e]
SGOT (Reitman-Frankel units/ml)	43.0 ± 13.3	69.6 ± 11.3[c]	46.4 ± 4.8
SGPT (Reitman-Frankel units/ml)	24.9 ± 6.9	18.9 ± 3.8	16.9 ± 3.3[e]
BUN (mg%)	19.8 ± 5.4	22.6 ± 3.8	19.4 ± 2.7
TP (gm%)	7.4 ± 0.6	6.5 ± 0.4[d]	6.5 ± 0.3[d]

[a] Monsanto Company, St. Louis, Missouri.

[b] Values expressed as mean ± 1 standard deviation.

[c] Difference with controls statistically significant: $p < .005$.

[d] Difference with controls statistically significant: $p < .01$.

[e] Difference with controls statistically significant: $p < .05$.

The livers of the animals from the two experimental groups were slightly enlarged and comprised 2.8 percent of their body weight while the control livers were 2.1 percent. Ultrastructural examination of the hepatic tissue demonstrated that the increase in size of the liver was due primarily to the proliferation of the smooth endoplasmic reticulum within the cytoplasm of the hepatic cells. The other cytoplasmic organelles and nuclei were unaltered. The liver homogenates of the experimental animals showed an increase in total RNA (Table II). However, due to the selected proliferation of the cytoplasmic endoplasmic reticulum, there was a decrease in the DNA content per gram of liver. Small modifications were also apparent in the enzyme activity of the microsomal fraction (Table III). There were significant decreases in glucose-6-phosphatase and esterase.

The percent recovery of Aroclor 1248 that was added to control tissues and samples of feces and urine was: feces, 98.7 ± 14.3; urine, 83.3 ± 6.3; kidney, 81.1 ± 12.3; brain, 82.2 ± 3.5; and liver, 94.8 ± 4.2.

The greatest percentage of the PCBs eliminated from the body occurred through biliary excretion into the gastrointestinal tract. By the 14th day, 5.60 percent of the original dose of the PCBs had been eliminated in the urine (0.38 mg) and feces (373 mg)

from the animal that had received 3.0 g per kg body weight of PCBs, and 5.75 percent (0.88 mg in the urine and 237 mg in the feces) of the dose had been eliminated by the monkey that received 1.5 g per kg body weight. The chromatogram of the standard PCBs

Table II. *Biochemical Alterations in the Liver of Monkeys Given PCBs (Aroclor 1248)*

	Control[a]	PCB dosage[a] (3 g/kg)	(1.5 g/kg)
mg protein/mg DNA	107 ± 28	107 ± 10	98.8 ± 11.0
mg DNA/100 mg liver	.346 ± .032	.266 ± .014[b]	.293 ± .031[c]
mg RNA/mg DNA	1.49 ± .08	2.19 ± .24[b]	1.99 ± .21[b]

[a] Values expressed as means ± 1 standard deviation.

[b] Difference with controls statistically significant: $p < .01$.

[c] Difference with controls statistically significant: $p < .05$.

Table III. *Hepatic Microsomal Alterations in Monkeys Given PCBs (Aroclor 1248)*[a]

	Control	PCB dosage 3 g/kg	1.5 g/kg
Aniline hydroxylation (mμmol *p*-aminophenol/30 min)[b]	11.1 ± 3.9	10.0 ± 2.0	13.6 ± 4.0
N-demethylation (mμmol formaldehyde/30 min)[b]	218 ± 36	257 ± 52	289 ± 89
Nitroreduction (mμmol *p*-aminobenzoate/hr)[b]	16.0 ± 3.3	21.5 ± 8.5	24.3 ± 9.8
Glucose-6-phosphatase (μmol PO_4/15 min)[b]	1.71 ± .37	1.13 ± .18[c]	1.47 ± .18
Esterase (μmol *p*-nitrophenol/min)[b]	3.44 ± .33	1.47 ± .18[d]	3.37 ± .62

[a] Values expressed as means ± 1 standard deviation.

[b] Per mg microsomal protein.

[c] Difference with controls statistically significant: $p < .001$.

[d] Difference with controls statistically significant: $p < .02$.

(Aroclor 1248) containing numerous peaks having retention times between 40 and 325 sec, differed markedly from the chromatograms of the urinary residue obtained on the fourth day which contained an isolated peak at 120 sec, and from that obtained from the urinary residue of the 14th day which contained a major peak at 120 sec (representing 93% of the material) and minor peaks at 280 and 400 sec. The chromatogram of the biliary residue contained five major peaks at 45, 80, 160, 300, and 355 sec (Figure 1, D).

At the time the animals were sacrificed on the fourth day, levels of PCBs in the brain and kidney were about half those found in the liver (Table IV). However, in tissues taken from the two animals that survived for two weeks there was a marked increase in liver PCBs and a decided decrease in levels of PCBs in the brain and kidney. Tracings of chromatograms of the liver residues taken at four days and 14 days after PCB administration are depicted in Figure 1, B and C. At four days there is a greater percentage of isomers and perhaps polar metabolites having longer retention times than those found in the

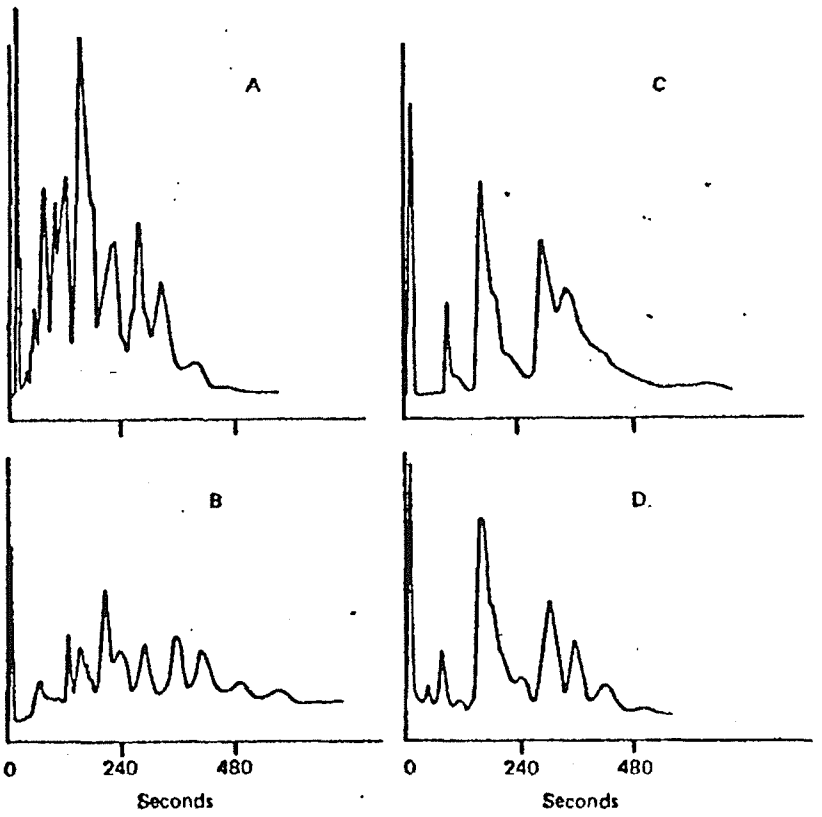

Fig. 1. GLC-EC tracings of (A) Aroclor 1248; (B) the residues found in the liver of a monkey 4 days, and (C) 14 days after PCB administration; and (D) the residue found in the gallbladder 14 days after PCB administration.

Table IV. *Levels of PCB (Aroclor 1248) in the Tissues of Monkeys*[a]

No. of animals	Days after exposure	Dose per kg body wt	Liver μg/gm	Kidney μg/gm	Brain μg/gm
5	4	1.5	24.9 ± 8.2	12.1 ± 4.4[b]	16.7 ± 7.3
5	4	3.0	52.9 ± 27.7	27.2 ± 8.2	28.1 ± 7.5
1	14	1.5	92.1	3.3	Not detectable
1	14	3.0	72.5	1.8	Not detectable

[a] Values expressed as means ± 1 standard deviation.

[b] Difference with liver value statistically significant: $p < .02$.

standard (Figure 1, A). At 14 days, the chromatograms of the hepatic residue contain four major peaks with a greater proportion of the material having longer retention times than those found in the standard.

Discussion

These data indicate that a large percentage of the PCBs administered in this experiment is readily absorbed from the gastrointestinal tract and is subsequently slowly eliminated from the body. These findings are in agreement with the percentage absorption of PCBs by rodents recently reported by Albro and Fishbein (1972). Probably as a result of the PCB metabolism and selective excretions, the chromatograms of the tissue residues and of the body excreta are modified from the chromatograms of the PCB standard. The residues of the liver samples which contained greater precentages of material with longer retention times suggest selective excretion of compounds having few chlorine atoms with the resultant hepatic residue consisting of a higher percentage of highly chlorinated and more polar compounds.

Of the tissues examined for PCB content at four days after administration of the material, the liver contained the highest levels. In the two animals that were allowed to survive for 14 days, the hepatic levels of PCBs were higher than the hepatic levels at four days, while the kidney and brain levels decreased to very low levels. High levels of PCBs were reported by Grant *et al.* (1971) to be present in the rat adipose tissue and it is assumed that the fat depots of the monkeys also contained high concentrations of the PCBs. An explanation for the PCB location within the liver at 14 days is that PCBs from other tissues, including that from brain, kidney, and adipose tissue, have been mobilized and subsequently selectively taken up by the liver.

During the period of reduced food intake following PCB administration, mobilization of lipid tissue containing the hydrophobic PCBs presumably occurred and resulted in the

release of fatty acids and PCBs into the circulatory system. The continued exposure of the liver to the compounds and a suggested preferential hepatic uptake resulted in the higher hepatic levels observed at 14 days.

The hematological changes that occurred in the animals during the four-day examination period were minimal. However, there were indications of slight modifications in the hemoglobin and hematocrit values of the experimental animals. The moderate leukocytosis and neutrophilia which occurred in this acute study are patterns observed in monkeys exposed to PCBs for longer periods. Monkeys fed lower levels of PCBs for a period of three months were anemic and developed a decided leukocytosis (Allen *et al.* 1973). The increase in circulating white blood cells may be a response to the gastric irritation that develops in animals following exposure to the PCBs. Although not particularly severe in the acute animals, ulcerations of the gastric mucosa and subsequent acute inflammatory responses may be sufficient to induce a leukocytosis. In addition, focal hemorrhage in the gastric mucosa of the more chronically affected animals was apparent. Hemorrhagic gastric ulceration would also account for a portion of the decrease in hemoglobin and hematocrit that occurred in the PCB-intoxicated animals.

There may also be an inhibiting effect of the PCBs on the hematopoietic tissue of the bone marrow and lymph tissue. The exact mechanism by which the PCBs produce these hypoplastic changes is not clear. Vos and De Roij (1972) have shown a decided decrease in antibody forming cells in the lymph nodes of guinea pigs that had been exposed to the PCBs. In addition, there was a decrease in circulating lymphocytes in the PCB-fed animals (Vos 1972).

The PCBs appear to have minimal immediate deleterious effects on liver function in the nonhuman primate, even when large doses are given. It has been shown in a number of animal species, including the monkey, that the PCBs are activators of microsomal drug metabolizing enzymes (Norback and Allen 1970; Allen and Abrahamson 1972). Degenerative changes in the hepatic cells and focal liver necrosis are observed in monkeys that have been fed high levels of PCBs for extended periods (Allen *et al.* 1973).

The lesions that developed in the gastric mucosa are possibly unique to the primate species. In the work that has been done on rodents (Allen and Abrahamson 1972), rabbits (Vos 1972), dogs (Keplinger *et al.* 1971), and other species (Vos and Koeman 1970), gastric hypertrophy, hyperplasia, or dysplasia of the mucosal epithelium have not been recorded. However, in human outbreaks of PCB intoxication, nausea and vomiting, both of which are consistent with gastric irritation, were a constant observation (Kuratsune 1969).

The presently reported research indicates that a single large dose of the PCBs to nonhuman primates is capable of producing injurious effects particularly to the gastric mucosa after short periods of time. Because of the high degree of absorption and subsequent tissue deposition, as well as the slow elimination from the body, large amounts of the PCBs remain in the body for long periods. Evidence suggests they are gradually

mobilized from the tissue stores, sequestered in the liver, and eliminated by biliary excretion. Mobilization of the PCBs ensures a continuing exposure of tissues to low level PCBs for an extended period.

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